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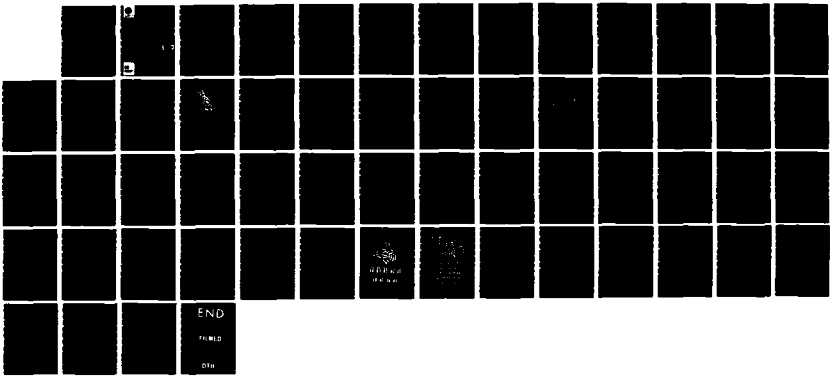

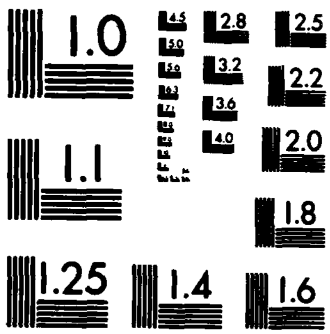
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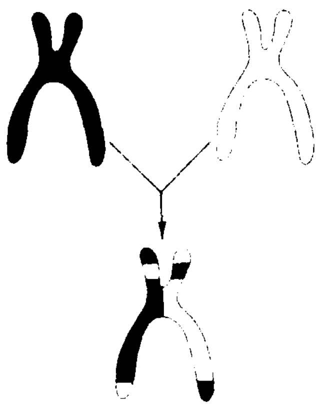

US Army Corps
of Engineers

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FIELD VERIFICATION PROGRAM
(AQUATIC DISPOSAL)

TECHNICAL REPORT D-85-1

APPLICATION OF SISTER CHROMATID EXCHANGE IN MARINE POLYCHAETES TO BLACK ROCK HARBOR SEDIMENT

LABORATORY DOCUMENTATION PHASE

by

Gerald G. Pesch, Cornelia Mueller, Carol E. Pesch,
James Heltshe Paul S. Schauer

Environmental Research Laboratory
US Environmental Protection Agency
Narragansett, Rhode Island 02882

January 1985
Final Report

Approved For Public Release; Distribution Unlimited

Prepared for DEPARTMENT OF THE ARMY
US Army Corps of Engineers
Washington, DC 20314-1000

and US Environmental Protection Agency
Washington, DC 20460

Monitored by Environmental Laboratory
US Army Engineer Waterways Experiment Station
PO Box 631, Vicksburg, Mississippi 39180-0631

The D-series of reports includes publications of the
Environmental Effects of Dredging Programs:

Dredging Operations Technical Support

Long-Term Effects of Dredging Operations

Interagency Field Verification of Methodologies for
Evaluating Dredged Material Disposal Alternatives
(Field Verification Program)

SUBJECT: Transmittal of Field Verification Program Technical Report Entitled "Application of Sister Chromatid Exchange in Marine Polychaetes to Black Rock Harbor Sediment; Laboratory Documentation Phase"

TO: All Report Recipients

1. This is one in a series of scientific reports documenting the findings of studies conducted under the Interagency Field Verification of Testing and Predictive Methodologies for Dredged Material Disposal Alternatives (referred to as the Field Verification Program or FVP). This program is a comprehensive evaluation of environmental effects of dredged material disposal under conditions of upland and aquatic disposal and wetland creation.

2. The FVP originated out of the mutual need of both the Corps of Engineers (Corps) and the Environmental Protection Agency (EPA) to continually improve the technical basis for carrying out their shared regulatory missions. The program is an expansion of studies proposed by EPA to the US Army Engineer Division, New England (NED), in support of its regulatory and dredging missions related to dredged material disposal into Long Island Sound. Discussions among the Corps' Waterways Experiment Station (WES), NED, and the EPA Environmental Research Laboratory (ERLN) in Narragansett, RI, made it clear that a dredging project at Black Rock Harbor in Bridgeport, CT, presented a unique opportunity for simultaneous evaluation of aquatic disposal, upland disposal, and wetland creation using the same dredged material. Evaluations were to be based on technology existing within the two agencies or developed during the six-year life of the program.

3. The program is generic in nature and will provide techniques and interpretive approaches applicable to evaluation of many dredging and disposal operations. Consequently, while the studies will provide detailed site-specific information on disposal of material dredged from Black Rock Harbor, they will also have great national significance for the Corps and EPA.

4. The FVP is designed to meet both Agencies' needs to document the effects of disposal under various conditions, provide verification of the predictive accuracy of evaluative techniques now in use, and provide a basis for determining the degree to which biological response is correlated with bioaccumulation of key contaminants in the species under study. The latter is an important aid in interpreting potential biological consequences of bioaccumulation. The program also meets EPA mission needs by providing an opportunity to document the application of a generic predictive hazard-assessment research strategy applicable to all wastes disposed in the aquatic environment. Therefore, the ERLN initiated exposure-assessment studies at the aquatic disposal site. The Corps-sponsored studies on environmental consequences of aquatic disposal will provide the effects assessment necessary to complement the EPA-sponsored exposure assessment, thereby allowing ERLN to develop and apply a hazard-assessment strategy. While not part of the Corps-funded FVP, the EPA exposure assessment studies will complement the Corps' work, and together the Corps and the EPA studies will satisfy the needs of both agencies.

SUBJECT: Transmittal of Field Verification Program Technical Report Entitled "Application of Sister Chromatid Exchange in Marine Polychaetes to Black Rock Harbor Sediment; Laboratory Documentation Phase"

5. In recognition of the potential national significance, the Office, Chief of Engineers, approved and funded the studies in January 1982. The work is managed through the Environmental Laboratory's Environmental Effects of Dredging Programs at WES. Studies of the effects of upland disposal and wetland creation are being conducted by WES and studies of aquatic disposal are being carried out by the ERLN, applying techniques worked out at the laboratory for evaluating sublethal effects of contaminants on aquatic organisms. These studies are funded by the Corps while salary, support facilities, etc., are provided by EPA. The EPA funding to support the exposure-assessment studies followed in 1983; the exposure-assessment studies are managed and conducted by ERLN.

6. The Corps and EPA are pleased at the opportunity to conduct cooperative research and believe that the value in practical implementation and improvement of environmental regulations of dredged material disposal will be considerable. The studies conducted under this program are scientific in nature and will be published in the scientific literature as appropriate and in a series of Corps technical reports. The EPA will publish findings of the exposure-assessment studies in the scientific literature and in EPA report series. The FVP will provide the scientific basis upon which regulatory recommendations will be made and upon which changes in regulatory implementation, and perhaps regulations themselves, will be based. However, the documents produced by the program do not in themselves constitute regulatory guidance from either agency. Regulatory guidance will be provided under separate authority after appropriate technical and administrative assessment of the overall findings of the entire program.

James Choromokos, Jr., Ph.D., P.E.
Director, Research and Development
U. S. Army Corps of Engineers

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Assistant Administrator for
Research and Development
U. S. Environmental Protection Agency

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Black Rock Harbor (Conn.)--Environmental aspects (LC)
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This report presents an evaluation of the applicability of the cytogenetic technique of sister chromatid exchange (SCE) to measure genotoxic effects of highly contaminated dredged material, and the degree of variability and reproducibility inherent in the procedure. This project is part of the US Environmental Protection Agency/Corps of Engineers Field Verification Program.

The SCE technique was applied to *Nephtys incisa*, an infaunal polychaete

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20 ABSTRACT (Continued).

dominant in the benthic community at the Central Long Island Sound disposal site. The SCE response was measured in *N. incisa* exposed to suspended particulate and bedded phases of Black Rock Harbor (BRH) sediment in the laboratory. *Neanthes arenaceodentata*, a surrogate species, was tested in parallel to *N. incisa*.

With the exception of one treatment in one experiment, the worm chromosomes were uniformly nonresponsive to BRH sediment. Replicate treatments within an experiment did not differ significantly for *N. arenaceodentata*. Differences between experiments and between species within an experiment were found. The reasons for the differences are not known. Differences in ability to metabolize polynuclear aromatic hydrocarbons, found in high concentrations in BRH sediments, is a likely but speculative reason. Clearly, additional research is needed before SCE could be used for routine testing.

This investigation is the first phase in developing field-verified bioassessment evaluations for the Corps of Engineers and the US Environmental Protection Agency regulatory program for dredged material disposal. This report is not suitable for regulatory purposes; however, appropriate assessment methodologies that are field verified will be available at the conclusion of this program.

PREFACE

This report describes work performed by the US Environmental Protection Agency, Environmental Research Laboratory, Narragansett, Rhode Island (ERLN), as part of the Interagency Field Verification of Testing and Predictive Methodologies for Dredged Material Disposal Alternatives Program (Field Verification Program (FVP)). This program is sponsored by the Office, Chief of Engineers, and the US Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss. The program objective of this interagency agreement is to verify existing predictive techniques for evaluating the environmental consequences of dredged material disposal under aquatic, wetland, and upland conditions. The aquatic portion of the FVP study is being conducted by ERLN, with the wetland and upland portions conducted by WES.

The principal investigators for this aquatic study were Dr. Gerald G. Pesch, Research Aquatic Biologist; Ms. Cornelia Mueller, Cytogenetic Technologist; Ms. Carol E. Pesch, Research Aquatic Biologist; Dr. Paul Schauer, Nutritionist; and Dr. James Heltshe, Statistician. The laboratory exposure system was designed by Dr. Paul Schauer. Mr. Michael Balboni, Dr. D. Michael Johns, and Ms. Ruth Gutjahr-Gobell assisted with collecting worms and conducting experiments. Data management and analysis were conducted by Mr. Jeffrey Rosen.

The EPA Technical Director for the FVP was Dr. John H. Gentile; Technical Coordinator was Mr. Walter Galloway; and Project Manager was Mr. Allan Beck.

Special thanks are due to Capt. Robert Alix and Dr. Anthony Calabrese of the National Marine Fisheries Service Laboratory, Milford, Conn., for field support and for use of their boat, the Shang Wheeler.

This study was conducted under the supervision of Drs. Richard K. Peddicord and Thomas M. Dillon, Environmental Laboratory (EL), Vicksburg, Miss. Mr. Charles C. Calhoun was Manager, Environmental Effects of Dredging Programs. Dr. John Harrison was Chief, EL. Commander and Director of WES during the conduct of this study and the preparation of this report was COL Tilford C. Creel, CE. Technical Director was Mr. F. R. Brown. The OCE Technical Monitors were Drs. John Hall and William L. Klesch.

This report should be cited as follows:

Pesch, G. G., et al. 1985. "Application of Sister Chromatid Exchange in Marine Polychaetes to Black Rock Harbor Sediment; Laboratory Documentation Phase," Technical Report D-85-1, prepared by US Environmental Protection Agency, Narragansett, R. I., for the US Army Engineer Waterways Experiment Station, Vicksburg, Miss.

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APPLICATION OF SISTER CHROMATID EXCHANGE IN MARINE POLYCHAETES TO BLACK ROCK HARBOR SEDIMENT

Laboratory Documentation Phase

PART I: INTRODUCTION

Background

1. Pollutants in coastal marine environments may affect the genetic constitution of exposed organisms by either causing shifts in gene pool composition through selective mechanisms or by acting directly on the genetic material to produce mutations. This report addresses the latter problem. There is evidence that some areas of the marine environment are contaminated with mutagens and carcinogens. Longwell and Hughes (1980) examined mackerel eggs sampled from the surface waters of the New York Bight. They observed mitotic chromosome irregularities, variable development rates as calculated by mitotic index, and differences in viability as estimated by early indicators of cell death. These effects were found in the more impacted areas of the Bight close to the coast and disposal grounds. In addition to the New York Bight, mutagens have been detected in other polluted marine environments (Parry et al. 1976; Payne et al. 1979). These observations suggest that genetic toxicants may be present in many polluted marine environments. Because the integrity of an organism's genes is essential for its well being, and genetic damage may accumulate from one generation to the next, genetic impairment may represent a long-term threat to populations of marine organisms.

2. The importance of genetic effects in marine pollution studies has been recognized only recently. The International Council for the

Exploration of the Seas dedicated a section of their 1979 Workshop on biological effects of marine pollution to genetics (Beardmore et al. 1980). They registered concern and recommended increased research efforts in this area.

3. To determine whether mutagenic compounds pose real threats to the marine environment, information is needed about the presence, distribution, and abundance of such toxicants in the marine environment, the degree of genetic damage suffered by marine organisms, and the ecological consequences of such damage. No single test can address all of these concerns. However, at present, no genetic tests are available for routine use in managing waste disposal in estuarine, coastal, and oceanic environments. Short-term tests are needed to identify mutagens and to evaluate complex mixtures. Long-term tests are needed also to investigate possible effects of somatic and germinal mutations on populations.

4. Several approaches are possible to detect and study genetic toxicants. Chemical analyses of environmental samples can be performed, but provide no information on the bioavailability of sediment-sorbed compounds. Futhermore, many classes of compounds are genetically active; therefore, these analyses are time-consuming and expensive. A simpler approach is to look for genetic damage in the exposed biota. Since cytogenetic techniques are sensitive and reasonably simple, it is recommended that genetic damage in marine organisms be determined by observing their chromosomes directly (International Atomic Energy Agency 1979; Kligerman 1980).

5. Polychaetes were selected for this study for several reasons. First, they have suitable karyotypes, that is, relatively large chromosomes

(Pesch and Pesch 1980a, and Appendix A). Second, they are easily handled and some species can be cultured to provide a continuous supply of experimental material. Third, polychaetes are benthic and of particular interest because sediments are often sinks for pollutants. Fourth, they are important food web species for commercially important fishes, and, therefore, may contribute to the trophic transfer of toxicants. Fifth, they are relatively sedentary so field-collected specimens would be representative of the area being sampled. Finally, some polychaetes are recognized as pollution indicators, particularly pollution associated with organic loading (Reish 1960; Wass 1967; Reish 1972).

6. The cytogenetic technique of choice is metaphase chromosome analysis. As cells divide, the chromosomes are drawn together in a condensed form easily visible with a light microscope. This is the metaphase stage of cell division. It is possible to arrest cells at this stage using the chemical colchicine. This permits the observation of chromosomes in a wide variety of tissues containing metaphase-arrested cells. Metaphase preparations allow accurate observation of chromosome structure and permit sister chromatid exchange (SCE) analysis. A sister chromatid exchange represents the breakage and reciprocol exchange of identical DNA material between the two sister chromatids of a chromosome. This was demonstrated originally by Taylor using tritium-labeled DNA (Taylor et al. 1957). The methods for differential staining for light microscopy were developed approximately 10 years ago (Figure 1) (Latt 1974; Perry and Wolff 1974). These new techniques transformed SCE from a limited, research tool to a tool which could be applied extensively to the study of environmental mutagenesis.

Figure 1. Sister chromatid exchange illustrated with the chromosomes of the polychaete N. incisa

7. The mechanism of SCE is not known and is the subject of much research. Several mechanisms have been postulated including altered replication of DNA past damaged bases (Shafer 1977), asynchrony in DNA synthesis between adjacent replicon clusters (Painter 1980), or a type of recombinational repair (Bender et al. 1974). As yet, there is no evidence to demonstrate conclusively a specific mechanism.

8. The usefulness of the SCE response as an indicator of DNA damage is based on both empirical and biological grounds. There is a wealth of SCE data showing dose responses to known mutagens in both in vitro and in vivo test systems (Latt et al. 1981). The response itself

indicates a direct effect on the DNA material; SCE is a visual consequence of mutagens that affect changes in the DNA helix. Because of our ignorance of the molecular mechanisms involved, we do not yet understand the significance or consequences of these DNA changes. However, SCE responses have been correlated with induced point mutations and may be useful as a quantitative indicator of mutagenesis (Carrano et al. 1978).

9. The SCE response has been recommended for environmental application by U.S. Environmental Protection Agency's Gene-Tox Program (Latt et al. 1981). Several studies have shown that SCE is a more sensitive method for detecting mutagens and carcinogens than the traditional chromosome and chromatid observations (Latt 1974; Perry and Evans 1975; Solomon and Bobrow 1975; Bloom 1978). The application of SCE to polychaetes has created a new tool that is both relevant and practical to study genetic problems in marine environments (Pesch et al. 1981). The SCE technique also makes it possible to measure the effect of toxicants on cell replication kinetics because differential staining of chromosomes permits easy identification of first, second, third, and subsequent replication cycle cells (Schneider et al. 1978). With the in vivo SCE assay, complex wastes can be tested under conditions which simulate real world situations.

10. The study reported herein applies the SCE technique to the polychaete _Neanthes arenaceodentata_. In earlier studies, _N. arenaceodentata_ exhibited a dose response to the known mutagen mitomycin C (MMC) at concentrations comparable to those that elicited responses in in vivo mammalian systems (Pesch & Pesch 1980b). Positive SCE responses in the worm have also been demonstrated for other known, direct-acting mutagens

such as 5-bromodeoxyuridine and methylmethanesulfonate, as well as for compounds that need metabolic activation such as benzopyrene, dimethylnitrosamine, and cyclophosphamide. These results imply that *N. arenaceodentata* can metabolize promutagens and suggest that the worm may be sensitive to a broad spectrum of genetic toxicants.

Objectives

11. This report is part of a comprehensive program sponsored by the U.S. Army Corps of Engineers (CE) and the U.S. Environmental Protection Agency (EPA) to evaluate the risk associated with various disposal options for dredged material. The approach being used in this Field Verification Program (FVP) is to evaluate and field validate assessment methodologies for predicting the environmental impacts of dredged material disposal in aquatic, upland, and wetland environments. The EPA Environmental Research Laboratory (ERLN), Narragansett, is responsible for the aquatic portion of the FVP.

12. There are three primary objectives in the aquatic portion of the FVP. The first objective is to demonstrate the applicability of the SCE technique to measure effects of dredged material, and to determine the degree of variability and reproducibility inherent in the procedure. The SCE technique will be applied to *Nephtys incisa*, an infaunal polychaete dominant in the benthic community at the Central Long Island Sound disposal site. The SCE response will be measured in *N. incisa* exposed to particulate and solid phase Black Rock Harbor sediment in the laboratory. *N. arenaceodentata* will be included in the laboratory phases of the FVP study as a surrogate species and will be tested in

parallel to *N. incisa*. This first objective is referred to as the Laboratory Documentation Phase of the FVP and is the subject of this report.

13. The second objective is to field verify the response observed in the laboratory and determine the accuracy of the laboratory prediction. Consequently, this portion of the study is referred to as the Field Verification Phase.

14. The third objective is to determine the degree of correlation of tissue residues resulting from the bioaccumulation of contaminants from dredged material and the response in SCE as observed in both the laboratory and the field. The second and third objectives will be combined in a final report for the FVP due in 1986.

PART II: METHODS AND MATERIALS

Overview

15. Two types of tests were conducted with polychaetes: short-term solid phase tests and short-term tests with both suspended particulate and solid (bedded) phase sediments. Both tests were 10-day flow-through tests. The solid phase tests used a gradation of highly contaminated Black Rock Harbor (BRH) sediment and Reference (REF) sediment from the reference station just south of the disposal site. Only one species, N. arenaceodentata, was tested in the short-term solid phase tests, whereas both species were included in the short-term suspended particulate/solid phase tests. N. incisa was not used in solid phase tests because the suspended particulate/solid phase tests included "worst" case solid phase exposure and because the cytogenetic technique for N. incisa was not perfected at the time solid phase tests were conducted. In the suspended particulate/solid phase tests, suspensions of either REF or BRH sediments were dosed in combination with a solid phase of 100 percent REF or 100 percent BRH sediment. This test, which combines the solid and particulate phase, is representative of the type of condition at the disposal site; however, the concentrations of suspended material used in the tests do not necessarily simulate actual field concentrations. These higher concentrations of suspended particulates were chosen to produce a dose response in the endpoint measurements in the short-term tests.

16. The tests described below generally follow methods prescribed in "Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians" (American Society for Testing and

and Materials 1980). Although the ASTM test methods were not specifically designed for polychaetes or sediment tests, they provide guidelines for experimental designs, water quality parameters, statistical analyses, and animal care, handling, and acclimation. The length of the tests, 10 days, was chosen based on the data provided in Pesch and Hoffman (1983). The 10 day LC_{50} values approached closely the incipient LC_{50} values; therefore, the cost of conducting 28-day tests was unwarranted unless chronic exposures were desired. Chronic exposures were not needed for purposes of laboratory documentation.

Sediment Collection and Preservation

17. Reference sediment for these studies was collected from the South reference site (41°7.95'N and 72°52.7'W), which is approximately 700 m south of the southern perimeter of the Central Long Island Sound disposal site (Figure 2). Reference sediment was collected with a Smith-McIntyre grab sampler (0.1 m^2) in August and December 1982 and May 1983 (collection I, II, and III, respectively). Sediment from each collection was returned to the laboratory, press sieved (wet) through a 2-mm mesh stainless steel screen, homogenized, and stored in polypropylene (collection I) or glass (collection II and III) containers at 4°C until used in experiments. Each container of material was coded with collection number and date, and jar number (Lake et al. 1984).

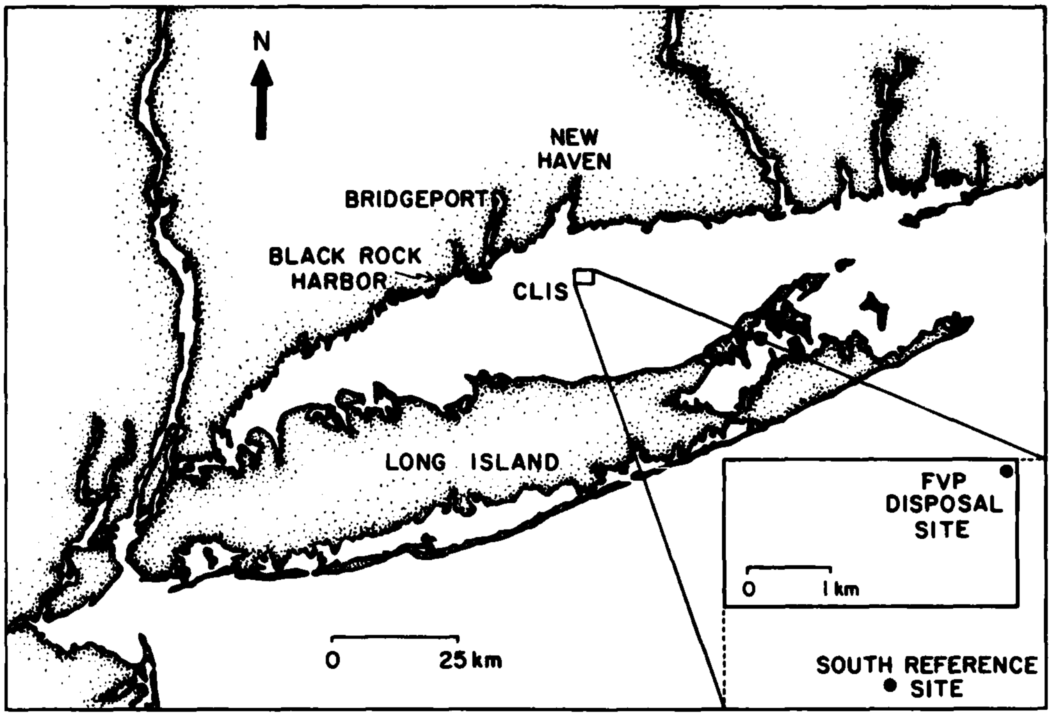

Figure 2. Central Long Island Sound disposal site and South reference site

18. Black Rock Harbor sediment was collected from 25 locations within the highly industrialized Black Rock Harbor (Bridgeport, Conn.) study area with a 0.1-m^2 gravity box corer to a depth of 1.21 m (Figure 3). The sediment was homogenized, distributed to barrels, and stored at 4°C. The contents of each barrel were homogenized, wet sieved through a 2-mm sieve, distributed to glass jars, and stored at 4°C until used in experiments. Samples of sediment were taken at various points in the collection, mixing, and distribution procedure for moisture content and chemical analysis (Lake et al. 1984).

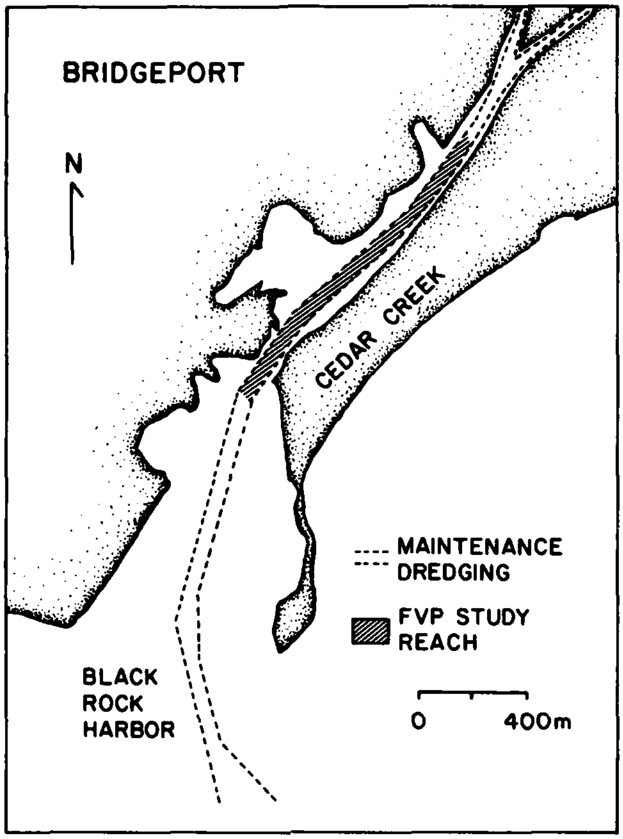

Figure 3. Black Rock Harbor, Connecticut, source of dredged material

Polychaete Collection, Culture, and Holding

19. Of the two species of polychaetes used, N. incisa and N. arenaceodentata, N. incisa is indigenous to the disposal area in Central Long Island Sound. They were collected with a Smith-McIntyre grab sampler (0.1 m^2) from the South reference site at various times in 1983 prior to the test periods and held in the laboratory for a short acclimation period (Appendix B). Neanthes arenaceodentata used in this study were from laboratory cultures at the same salinity and temperature used in these tests. Details of culture methods and conditions have been published

by Reish (1980). Nutritional requirements were determined by Schauer and Pesch (In Press). For both species, all tests were conducted with juvenile worms (Appendix B).

Test System

20. The suspended sediment experimental system consisted of three modules: the controlled dosing system, the dilution and distribution system, and the test chambers. Two identical dosing systems, one for REF and one for BRH, provided a constantly recirculating source of concentrated sediment slurry (in seawater) passing by a three-way valve that leads to the dilution and distribution system (Figure 4). Argon gas was added to the reservoir of the dosing system to minimize oxidation of the slurry. The three-way valve was controlled by a microprocessor programmed to deliver a pulse of slurry at periodic intervals. In the dilution and

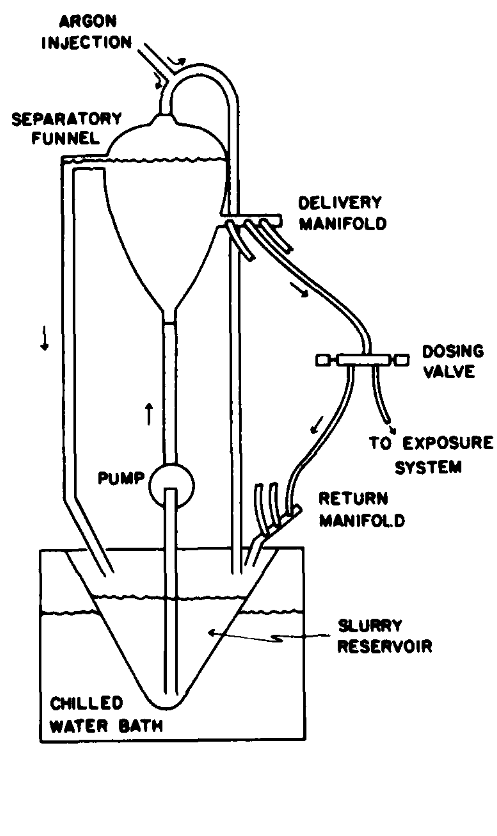

Figure 4. Sediment dosing system with chilled water bath and argon gas supply

distribution system, the concentrated slurry was mixed with seawater to the proper concentration of suspended solids and distributed to the individual test chambers. Actual concentration of suspended particulates in the test chambers was determined (by dry weights) periodically (Lake et al. 1984).

21. Two types of tests were conducted: a solid phase (bedded sediment) test without suspended particulates and a solid phase test with suspended particulates. The tests were conducted in glass crystallizing dishes (150 by 75 mm). Each dish contained a smaller glass crystallizing dish (60 by 35 mm) in the center of the larger dish. A Teflon®-coated stir bar was placed in the small dish in the center, which received the inflow water, to keep the particulate material in suspension (Figure 5). In the solid phase tests, the stir bar was omitted and the in-flow water was sandfiltered seawater. The inflow water flowed out of the central dish over the sediment surface, and overflowed the edge of the crystallizing dish. Each dish contained 400 ml of sediment (2.5 to 3.5 cm deep). The exposure concentrations used in the solid phase tests were: 100, 75, 50, 25, and 0 percent BRH (100 percent REF). The mixtures of the two sediments were made volumetrically, mixed thoroughly, and then distributed to the exposure chambers (Rogerson et al. 1984). Worms were fed prawn flakes, 30 mg every other day, directly onto the sediment surface during the tests. Two solid phase tests without suspended particulates were conducted with N. arenaceodentata. All tests included a mitomycin C treatment (5 by 10^{-6} M). Mitomycin C is a known mutagen and is included as a test standard for the SCE response (Pesch et al. 1981). The worms for the mitomycin C (MMC) treatment were exposed to

reference sediment for 9 days, then were sieved and exposed without sediment to MMC in seawater for the last day of the 10-day test.

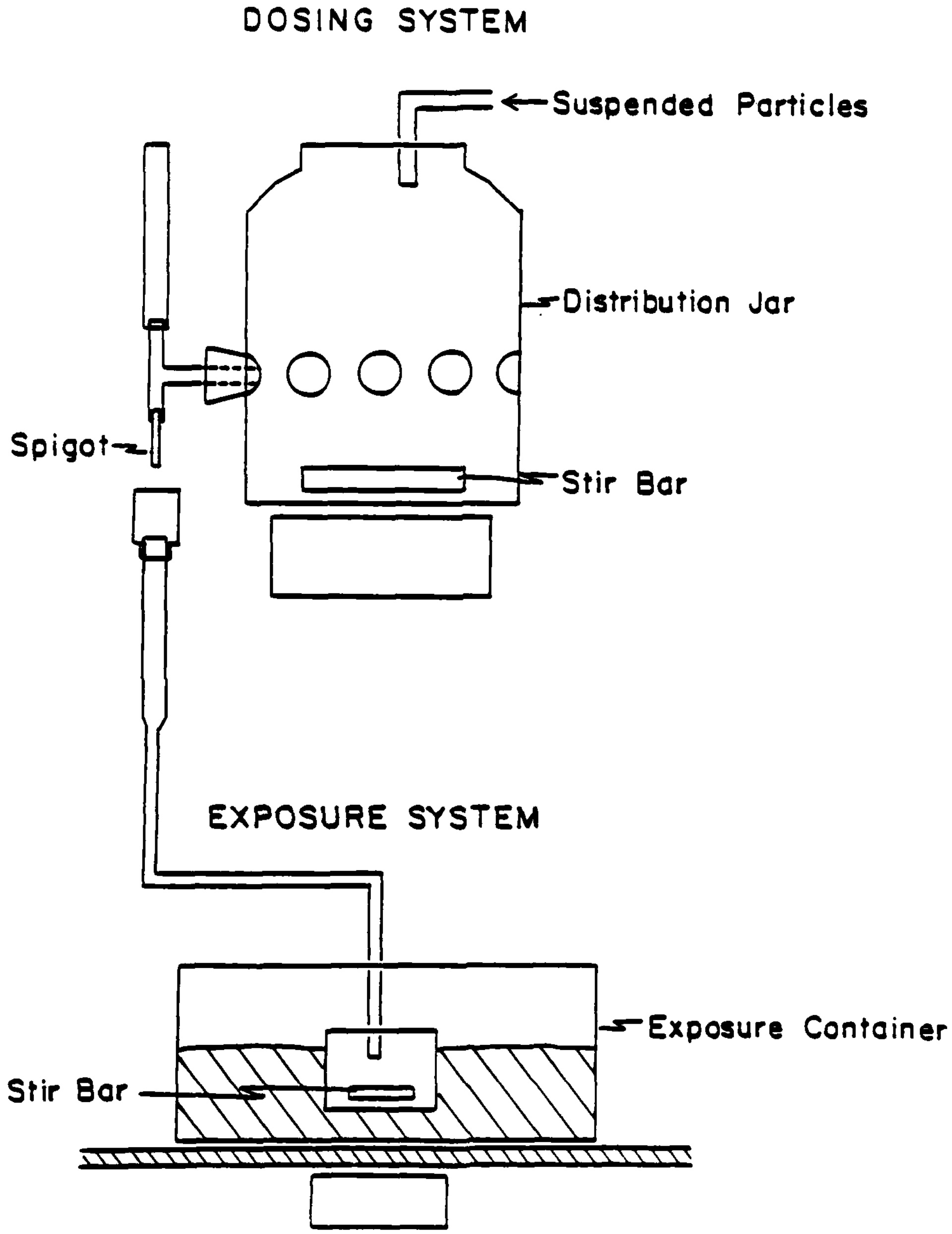

Figure 5. Schematic of the distribution and dosing system used to expose juvenile *N. incisa* and *N. arenaceodentata* to reference and BRH sediment

22. Exposure conditions for the solid phase portion of the suspended particulate tests were 100 percent REF or 100 percent BRH. These two solid phase exposure conditions in combination with the two suspended sediment exposures, REF or BRH at about 200 mg/ℓ (dry weight), gave a total of 4 treatments. The worms were fed prawn flakes (ADT-Prime, Aquatic Diet Technology, Brooklyn, N.Y.) in a suspension of seawater, which was pumped by peristaltic pump into the distribution chamber of the dosing system. The amount fed was 127 mg per test chamber per day. This amount of food was determined in prior feeding studies with _N. incisa_. Three identical suspended particulate tests were conducted with _N. incisa_ and two tests with _N. arenaceodentata_.

23. During the tests, all dishes were examined daily for the appearance of any worms on the surface of the sediment. Stressed worms will come to the surface of the sediment and remain there. On the last day of the test, observations were made on the burrows visible through the sides of the dishes and the depth of suspended material deposited on top of the solid phase was measured. Then the sediment was sieved (0.335 mm mesh) and the worms retrieved and counted.

24. All tests were conducted with sand-filtered Narragansett Bay seawater at 20°C and approximately 30 ppt salinity. Flow rates were about 35 ml/min. The photoperiod was a 14:10 hr light-dark cycle.

Chromosome Labeling

25. In order to facilitate the SCE observations, the chromosomes must be differentially stained. The differential staining is a consequence of labeling the chromosomes with the base analog 5-bromodeoxyuridine (BrdU)

for two cell cycles (Latt 1982). Because the labeling phase must include two cell cycles, the time needed will vary according to growth rate. Therefore, this time may differ by species and age of an organism. In this study, these times were determined empirically.

26. The labeling phase was done subsequent to the exposure phase. Because of different holding requirements, the labeling conditions were different for the two species of worms. However, both species were exposed to the same concentration of the label BrdU, 3 mg/ℓ. Neanthes arenaceodentata were labeled in 1ℓ of filtered seawater (30 ppt salinity) in crystallizing dishes at 20°C in the dark. Each treatment had approximately 15 worms and these were fed 30 mg of prawn flakes every other day. The labeled seawater was renewed every other day. Labeling lasted from 4 to 10 days depending on the size of worms. The larger worms were labeled longer because of longer cell cycle times. Colchicine (0.05 percent) was added to the seawater for the last 5 hr of the labeling period to arrest cell division.

27. To maintain healthy worms, N. incisa must be held in sediment; therefore, they were placed in clean, fine-grained sand with 2ℓ of filtered, labeled seawater (30 ppt). They were held at 20°C in subdued light. Each treatment had approximately 15 worms and these were fed 30 mg of prawn flakes every other day. Each time food was added, 2.3 g of reference sediment was added also, because in prior feeding studies, the presence of suspended sediment was found to enhance growth. The sediment was maintained in suspension by gentle aeration of the seawater. The labeled seawater was renewed every other day. The worms were labeled

for 10 days after exposure to sediments. Colchicine (0.05 percent) was added to the seawater for the last 15 hr of the labeling period.

Slide Preparation and Staining

28. The following procedure for slide preparation was adapted from Kligerman and Bloom (1977). The worms were removed from the labeling treatments and placed in 100 ml of 0.075 M solution of potassium chloride for 1 hr. They were then fixed in three changes of cold ethanol-acetic acid 3:1) for 0.5 hr each. Fixed worms were placed individually in a clean well slide and 1 ml of 60 percent acetic acid was added. The worm was macerated for approximately 1 min or until the tissue appeared translucent. The material was then drawn up into Pasteur pipettes and applied to clean, hot (45°C) slides. Excess acetic acid was immediately removed from the slides. This procedure produced a monolayer of separated cells on each slide.

29. Slides were stained according to a procedure recommended by Bloom.* Slides with BrdU-labeled chromosomes were stained with 225 μg ml^{-1} of 33258 Hoechst stain for 10 min (several drops placed on slide and coverslip added), rinsed in distilled water, and air dried. The slide was then wet mounted using an excess of McIlvaine's buffer (0.1 M citric acid, 0.2 M disodium phosphate) at pH 8.0 and placed between two black lights for 60 min. The slide was then rinsed in distilled water, air dried briefly, and stained with 2 percent Giemsa in deionized water

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* Personal Communication, S.E. Bloom, 1981, Cornell University, Ithaca, New York.

for 7 min. The slide was rinsed in distilled water, air dried, soaked in xylene, and mounted in Coverbond. Observations were made with a research microscope at 1250X, oil immersion.

Data Collection and Analysis

30. The SCE observations were made on 25 second-division (metaphase stage) cells for each treatment unless otherwise noted. Cells were selected under low power, then counted under high power. For each treatment, the individual worms were screened sequentially. Counting continues until a total of 25 cells were counted regardless of the number of individual worms. This assumes that organism-to-organism variance was small compared to within organism variance. This has been tested for these data and found to be true.

31. The data were examined to see whether criteria were met for parametric statistical analysis or whether data transformation was necessary. All of the SCE data in this report were transformed to log 10 (SCE/chromosome + 0.1) prior to statistical analysis. The 0.1 is added because log (0) is undefined. The means and standard error of the untransformed data are included in Appendix B. Statistical procedures used in this report are from Snedecor and Cochran (1980). Photographs were taken at 1000X through a green filter using high contrast copy film.

PART III: RESULTS AND DISCUSSION

Statistical Properties of SCE Data

32. Parametric statistical procedures used to analyze data (e.g., analysis of variance) assume that the data are normally distributed and that the variances are equal for all treatments being compared. These fundamental assumptions have been examined for SCE data in two different test systems. Dixon and Clarke (1982) reported that for adult blue mussels, *Mytilus edulis*, SCE data follow a Poisson distribution for individual animals. However, SCE counts for single cells from randomly selected animals within each treatment indicated the presence of variability between animals. They corrected for these problems by summing the SCE/cell counts for each animal and then applying a square root transformation. This stabilized the variance for all treatments and permitted parametric statistical analysis of the data. Carrano and Moore (1982) have provided an extensive evaluation of SCE data from human lymphocytes. They found that no single family of probability distributions could be used to describe all of their data sets. Consequently, they applied a nonparametric test for statistical comparisons.

33. The statistical attributes of SCE data for *N. arenaceodentata* were examined using results from the larval bioassay test (Pesch et al. In Press). This section is based on these data. All baseline observation data were pooled and the variable X = SCE's/chromosome was considered. There were 447 metaphase cell observations in this data set and their frequency distribution was skewed to the right and clearly did not follow a normal distribution. Based upon these results, a transformation of

the SCE/chromosome data prior to statistical analysis seemed appropriate. To determine the most appropriate transformation, the mean SCE's/chromosome were plotted for all experimental treatments (63 treatments, 25 observations/treatment) against their standard deviation. There was a very good relationship between standard deviation and mean, further evidence that a transformation of the data was appropriate. Although Latt et al. (1981) mention several possible transformations, it was concluded that the log 10 transformation is appropriate because it removes the relationship between mean and standard deviation; also, means based upon sample sizes as small as 5 are normally distributed (Pesch et al. In Press).

34. Another concern is the statistical sensitivity of the *N. arenaceodentata* SCE assay to detect an increased SCE response over baseline. The sensitivity of the method is evaluated by calculating the associated statistical power of the test to detect an increase in SCE frequency. The power of a statistical test is defined to be one minus the probability of making a type II error (false negative). The probability of type I error (false positive) is the preselected level of significance of a statistical test, typically $P = 0.05$. The probability of type II error (and power) is a function of the differences to be detected, the variance of the mean response, and sample size. A twofold increase over baseline SCE frequencies is recommended to indicate a positive response (Latt et al. 1981). To increase sample size is labor intensive with the *N. arenaceodentata* SCE assay. Therefore, the simplest approach to increase the power of the test is to decrease the variance of the mean baseline response. Carrano and Moore (1982) examined this

problem thoroughly and concluded that in the human lymphocyte system the "major source of inherent variation in baseline SCE frequencies can be attributed to the amount of BrdU present in the culture medium relative to the number of lymphocytes initially added." They dealt with this problem by selecting, as baseline, a BrdU concentration that was in the middle of the plateau region of the dose response curve. The plateau of the curve is by definition a region of minimum slope, so variations in BrdU concentration or cell numbers have minimal impact on baseline SCE frequencies.

35. BrdU elicits a dose response in N. arenaceodendata as in other test systems (Carrano et al. 1980; Wolff and Perry 1974). However, with N. arenaceodentata the variance associated with the mean response (log scale) increases by a factor of 2 in the plateau region of the curve. Therefore, a baseline BrdU concentration selected from the plateau region would reduce greatly the power of a statistical test to detect increases above baseline. An examination of the data indicates that the sensitivity of the assay is enhanced greatly using a baseline concentration of 3 mg/ℓ BrdU vs. 15 mg/ℓ (Pesch et al. In Press). The accepted protocol (Latt et al. 1981) of using 25 counts per replicate is sufficient to detect a doubling of the mean response using 3 mg/ℓ BrdU as a baseline concentration. The power to detect this doubling is 0.9 using a two-sample t-test with variance estimated from baseline data. A baseline concentration of 3 mg/ℓ BrdU allows for a more sensitive statistical test because of a lower variance of the mean.

Applicability of SCE Technique

36. *Neanthes arenaceodentata* were exposed to a series of the test sediments in solid phase only (Appendix B). This experiment was replicated as a randomized block design. In no case were the SCE frequencies of any treatment different from any other treatment (Table 1).

Table 1

Sister Chromatid Exchange/Chromosome Response for Two Replicate Experiments, Solid Phase Dosing of N. arenaceodentata

Treatments % BRH*	Exp. 1	Exp. 2	Pooled Data
0	−0.803 ± 0.049**(25)†	−0.802 ± 0.041(25)	−0.802 ± 0.032(50)
25	−0.783 ± 0.047(25)	−0.806 ± 0.043(25)	−0.794 ± 0.031(50)
50	−0.714 ± 0.049(25)	−0.826 ± 0.043(25)	−0.769 ± 0.033(50)
75	−0.716 ± 0.040(25)	−0.870 ± 0.031(25)	−0.793 ± 0.028(50)
100	−0.807 ± 0.045(25)	−0.789 ± 0.041(25)	−0.798 ± 0.030(50)
MMC††	−0.534 ± 0.065(25)	ND‡	

Note: Data expressed as log 10 of means.
* Percentage Black Rock Harbor sediment mixed volume for volume with Reference sediment.
** Standard error of the mean.
† Number in parentheses is sample size (N).
†† MMC is a known mutagen and is included as a test standard for the SCE response.
‡ ND = no data.

37. The pattern of no SCE response to BRH sediment continued for the particulate phase/solid phase experiments with *N. arenaceodentata* (Table 2). Again, in replicate experiments (ramdomized block design), the SCE frequencies of any treatment were not different from any other treatment.

Table 2

Sister Chromatid Exchange/Chromosome Response for Replicate Experiments,

Particulate Phase/Solid Phase Dosing of N. incisa and N. arenaceodentata

Treatment	Exp. 1	Exp. 2	Exp. 3	Pooled Data
		N. incisa		
B/R*	−0.450 ± 0.050**(25)†	−0.414 ± 0.055(12)	−0.194 ± 0.056(19)	.355 ± 0.035(56)
B/B	−0.543 ± 0.050(26)	−0.513 ± 0.054(15)	−0.449 ± 0.053(25)	−υ.500 ± 0.031(66)
R/R	−0.494 ± 0.145 (7)	−0.512 ± 0.049(25)	−0.482 ± 0.123 (9)	−0.502 ± 0.046(41)
R/B	−0.608 ± 0.056(25)	ND††	−0.403 ± 0.055(24)	−0.508 ± 0.041(49)
MMC‡	0.109 ± 0.275 (3)	−0.095 ± 0.051(20)	0.144 ± 0.246 (3)	−0.044 ± 0.056(26)
		N. arenaceodentata		
R/B	−0.644 ± 0.058(25)	−0.710 ± 0.060(25)		−0.676 ± 0.042(50)
B/R	−0.656 ± 0.053(25)	−0.705 ± 0.040(25)		−0.680 ± 0.033(50)
R/R	−0.689 ± 0.053(25)	−0.683 ± 0.048(25)		−0.685 ± 0.036(50)
B/B	−0.719 ± 0.048(25)	−0.655 ± 0.056(25)		−0.686 ± 0.037(50)
MMC‡	−0.348 ± 0.058(25)	−0.358 ± 0.054(25)		−0.353 ± 0.039(50)

Note: Data expressed as log 10 of means

* R = reference sediment, B = Black Rock Harbor sediment, numerator = particulate phase, denominator = solid phase.

** Standard error of the mean.

† Number in parentheses is sample size (N).

†† ND - no data.

‡ MMC is a known mutagen and is included as a test standard for the SCE response.

38. The particulate phase/solid phase experiments were replicated three times with _N. incisa_ using a randomized block design. Worms used in these replicate experiments were from different collection trips to the South reference site. In all three experiments, the B/R treatment had the highest SCE frequencies (Table 2). In the pooled data, the SCE frequencies for the B/R treatment were significantly higher ($P < 0.01$, ~ 50 percent higher) than any other treatment. There were no significant differences among the other treatments.

39. These findings raise several questions. Would we expect to see an increase in SCE frequencies due to Black Rock Harbor sediment? If so, why only in the B/R treatment? Why not in the presumably worst case treatment, B/B? Also, why would there be a difference in the response for the two species tested?

40. Black Rock Harbor sediment contains high levels of polynuclear aromatic hydrocarbons (Rogerson et al. 1984). Such compounds are known to be mutagenic; however, they require metabolic activation to become so (Bresnick 1976). The metabolic activation of hydrocarbons is mediated by a mixed-function oxygenase (MFO) system. Polychaetes have been shown to have active, inducible MFO systems (Lee and Singer 1980; Lee et al. 1979). Pesch et al. (1981) demonstrated that the polynuclear aromatic hydrocarbon, benzo(a)pyrene, caused increased SCE frequencies in the polychaete _N. arenaceodentata_.

41. The required induction of an MFO system provides a speculative answer for the remaining questions. Johns and Gutjahr-Gobell (in preparation) have studied the impact of BRH sediment on the bioenergetics of _N. incisa_. These studies were conducted simultaneously in the same

system as the chromosome studies. They found that the presumably worst case treatment (B/B) caused N. incisa to lose weight during the experiments, whereas the B/R treatment was not significantly different from R/R treatment for any of the measures of energetic effects. Because metabolic activation of the contaminants is needed, the worst case worms may be protected by their reduced metabolic activity. The worms in the B/R treatment did well metabolically and thus may have been susceptible. Since no data are available on MFO activity in these worms, this explanation of the observed SCE response is speculative. The ability to metabolize polynuclear aromatic hydrocarbons is centrally important to the toxicity of such compounds. Clearly, this is an area where additional research is needed.

42. The difference between species has several possible explanations. Pesch et al. (1981) demonstrated that N. arenaceodentata has an active MFO system. It may be that N. incisa has a more effective MFO system. More likely, the N. incisa (field collected vs. laboratory cultured) had been exposed previously to polynuclear aromatic hydrocarbons (PAH). These worms (N. incisa) were collected from the reference site. There are measurable levels of PAH (Rogerson et al. 1984) at the reference site. This low level exposure may induce increased MFO activity. Thus, the two species may have had considerably different levels of MFO activity during the experiments. Such a difference could explain the difference in SCE response.

Variability and Reproducibility of Data

43. Table 3 presents results from a separate experiment comparing duplicate treatments within a particulate phase/solid phase dosing experiment of *N. arenaceodentata*. The particulate phase concentration was 25 mg/ℓ (Appendix B).

Table 3

Sister Chromatid Exchange/Chromosome Response for Duplicate Treatments Within an Experiment, Particulate Phase/Solid Phase Dosing of *N. arenaceodentata*

Treatment	log Mean
R/R*	-0.753 ± 0.052**(25)†
R/R	-0.789 ± 0.044(25)
B/B	-0.723 ± 0.052(25)
B/B	-0.809 ± 0.042(23)

Note: Data expressed as log 10 of means.

* R = reference sediment, B = Black Rock Harbor sediment, numerator = particulate phase, denominator = solid phase.

** Standard error of the mean.

† Number in parentheses is sample size (N).

44. The assumed worst case, B/B, was duplicated and compared to duplicates of R/R. There were no statistically significant differences among any of the four treatments. Replicates of the same conditions were not significantly different from one another. These results indicate that between-treatment variance is low.

45. With *N. arenaceodentata*, replicate experiments were conducted with solid phase dosing and with particulate phase/solid phase dosing.

The particulate phase/solid phase replicates were not statistically different from each other; however, the overall means of the solid phase replicates differed at the P = 0.05 level with _N. arenaceodentata_.

46. The particulate phase/solid phase dosing experiment was replicated three times with _N. incisa_ (Table 2). Two of the experiments (Exps. 1 and 2) were not significantly different from each other, nor were there any significant differences among treatments. The overall mean for the third experiment (Exp. 3) was significantly higher than the overall means for the other two experiments. Also, the B/R treatment values were significantly higher than the other treatments within the third experiment. These results indicate a lack of reproducibility among experiments for _N. incisa_. However, the R/R (control) treatment was not different among the three experiments. The most likely explanation of the experiment-to-experiment differences is a biological difference among the three collections of worms. The collection site (South reference site) has low levels of PAH's (Rogerson et al. 1984) and has old patches of dredged material (personal observation). Prior exposure to PAH's could influence the inducible MFO system in the worms. The level of MFO activity in the worms could influence their SCE response to BRH sediment. Since no MFO data are available, this explanation of the observed SCE responses is speculative.

47. All statistical comparisons involving replicate experiments were made using a randomized block design. In this way, differences among experiments (blocks) were accounted for in determining differences among treatments. Using this method, the B/R treatment was significantly higher

than all other treatments with *N. incisa*. The other treatments were not significantly different from each other.

PART IV: CONCLUSIONS

48. The objective of the Laboratory Documentation Phase of the Field Verification Program for cytogenetic studies was to determine the applicability of sister chromatid exchange to measuring contaminated dredged material effects, and to determine the degree of variability and reproducibility inherent in the procedure. A major challenge was to apply the SCE technique to *Nephtys incisa*. This was done successfully. *Nephtys incisa* and the surrogate species, *Neanthes arenaceodentata*, were then used in parallel to test the effect of contaminated Black Rock Harbor sediment on the sister chromatid exchange response. With the exception of one treatment in one experiment (B/R, Exp. 3, Table 2, *N. incisa*), the worm chromosomes were uniformly nonresponsive to BRH sediment.

49. Replicate treatments within an experiment did not differ significantly for *N. arenaceodentata* (Table 3). Differences between experiments and between species within an experiment were found (Tables 1 and 2). The reasons for these differences are not known. Differences in ability to metabolize polynuclear aromatic hydrocarbons, found in high concentrations in BRH sediments, is a likely but speculative reason. Clearly, additional research is needed before SCE could be used for routine testing.

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APPENDIX A: KARYOTYPES

Chromosome complements (karyotypes) of *Neanthes arenaceodentata* and *Nephtys incisa* are shown in Figures A1 and A2, respectively.

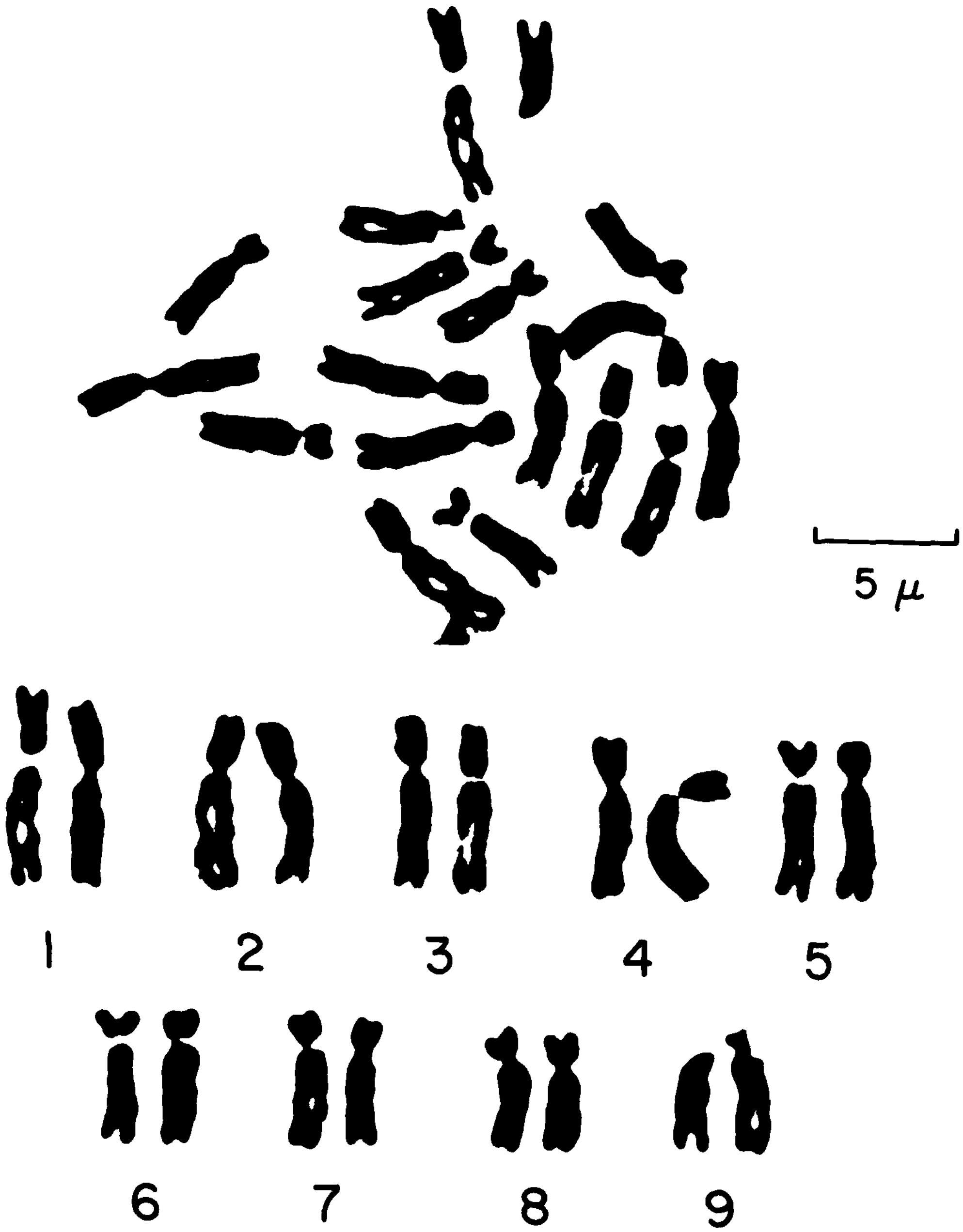

Figure A1. Chromosomes of Neanthes arenaceodentata

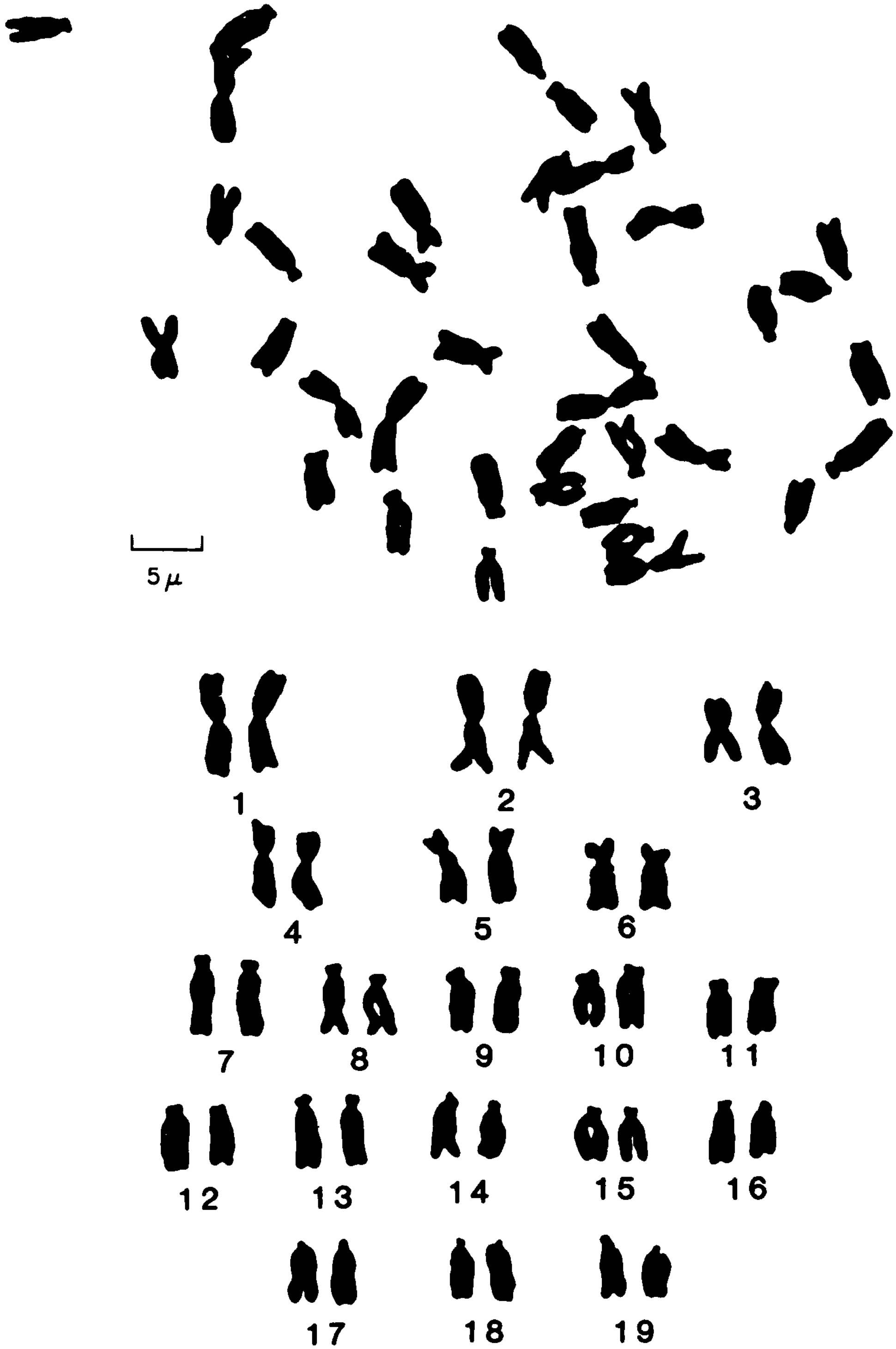

Figure A2. Chromosomes of <u>Nephtys incisa</u>

APPENDIX B: DATA SHEETS

Tables B1 through B8 are the laboratory data sheets for the experiments included in this report. The data sheets are identified by table number and experiment number as these numbers appear in the text of the report for each species tested.

TABLE B1

LABORATORY WORM DATA SHEET
COE/ERLN FVP

STUDY PLAN: 4 INVESTIGATOR: PESCH ET. AL.

EXPERIMENT DESCRIPTION: SOLID DATE OF TEST: 830104

EXP. NUMBER: 1 (Table 1) SPECIES: NEANTHES ARENACEODENTATA

** EXPERIMENTAL CONDITIONS **

TEMPERATURE: 20.00 DEGREES CENTIGRADE RANGE: 19.00 - 21.00
SALINITY: 31.00 PARTS PER THOUSAND RANGE: 30.00 - 32.00
EXPOSURE DURATION: 10 DAYS
PHOTOPERIOD: 9 HOURS
FLOW RATE: 68 MLS/MIN VOLUME ADDITIONS/DAY 130
NUMBER OF ANIMALS/REPLICATE: 25 NUMBER OF REPLICATES/TREATMENT: 1
ANIMAL'S LIFE STAGE: JUVENILE AGE: 53 DAYS
SIZE: 0.7 +/- 0.36 MG DRY WT RANGE:
CONTROLS: 100% REF
FOOD: PRAWN FLAKE DIRECTLY TO DISH
ANIMAL SOURCE: CULTURE
COLLECTION TEMPERATURE: DEGREES C COLLECTION SALINITY: PPT
ACCLIMATION:
SEDIMENT SOURCE: BARREL OR COLLECTION/JAR NUMBER
SOLID REFERENCE: I/- SOLID BRH: LL/19
SUSPENDED REFERENCE: SUSPENDED BRH:

SAMPLE NUMBER	EXPOSURE CONCENTRATIONS (1) NOMINAL	MEASURED	OXYGEN MG/L	MEAN LOG(SCE/CHROM+.1)	MEAN SCE/CHROMO
400039	100% REF			-0.80	0.09 ± 0.03*
400040	25% BRH			-0.78	0.09 ± 0.02
400041	50% BRH			-0.71	0.12 ± 0.02
400042	75% BRH			-0.71	0.11 ± 0.01
400043	100% BRH			-0.80	0.07 ± 0.02
400153	MMC			-0.53	0.28 ± 0.06

* Standard error of the mean.

TABLE B2

LABORATORY WORM DATA SHEET
COE/ERLN FVP

STUDY PLAN: 4 INVESTIGATOR: PESCH ET. AL.

EXPERIMENT DESCRIPTION: SOLID DATE OF TEST: 830531

EXP. NUMBER: 2 (Table 1) SPECIES: NEANTHES ARENACEODENTATA

** EXPERIMENTAL CONDITIONS **

TEMPERATURE: 21.00 DEGREES CENTIGRADE RANGE: 21.00 - 22.00
SALINITY: 30.00 PARTS PER THOUSAND RANGE: 28.00 - 30.00
EXPOSURE DURATION: 10 DAYS
PHOTOPERIOD: 15 HOURS
FLOW RATE: 64 MLS/MIN VOLUME ADDITIONS/DAY 122
NUMBER OF ANIMALS/REPLICATE: 15 NUMBER OF REPLICATES/TREATMENT: 1
ANIMAL'S LIFE STAGE: JUVENILE AGE: 62 DAYS
SIZE: +/- MG DRY WT RANGE: NOT AVAILABLE
CONTROLS: 100% REF
FOOD: PRAWN FLAKE DIRECTLY TO DISH
ANIMAL SOURCE: CULTURE
COLLECTION TEMPERATURE: DEGREES C COLLECTION SALINITY: PPT
ACCLIMATION:
SEDIMENT SOURCE: BARREL OR COLLECTION/JAR NUMBER
SOLID REFERENCE: II/52 SOLID BRH: LL/35
SUSPENDED REFERENCE: SUSPENDED BRH:

SAMPLE NUMBER	EXPOSURE CONCENTRATIONS (1) NOMINAL	MEASURED	OXYGEN MG/L	MEAN LOG(SCE/CHROM+.1)	MEAN SCE/CHROMO
400049	100% REF			-0.80	0.07 ± 0.01*
400050	25% BRH			-0.80	0.07 ± 0.01
400051	50% BRH			-0.82	0.06 ± 0.01
400052	75% BRH			-0.87	0.04 ± 0.01
400053	100% BRH			-0.78	0.08 ± 0.01

* Standard error of the mean.

TABLE B3

LABORATORY WORM DATA SHEET

COE/ERLN FVP

STUDY PLAN: 4 INVESTIGATOR: PESCH ET. AL.

EXPERIMENT DESCRIPTION: SUSPENDED DATE OF TEST: 830816

EXP. NUMBER: 1 (Table 2) SPECIES: NEPHTYS INCISA

** EXPERIMENTAL CONDITIONS **

TEMPERATURE: 21.00 DEGREES CENTIGRADE RANGE: 21.00 - 22.00
SALINITY: 30.00 PARTS PER THOUSAND RANGE: 30.00 - 31.00
EXPOSURE DURATION: 13 DAYS
PHOTOPERIOD: 14 HOURS
FLOW RATE: 35 MLS/MIN VOLUME ADDITIONS/DAY 67
NUMBER OF ANIMALS/REPLICATE: 14 NUMBER OF REPLICATES/TREATMENT: 1
ANIMAL'S LIFE STAGE: JUVENILE AGE: DAYS
SIZE: +/- MG DRY WT RANGE: .458-4.662 MG
CONTROLS: 200 MG/L REF/REF
FOOD: PRAWN FLAKE SUSPENSION
ANIMAL SOURCE: SOUTH REFERENCE SITE, LONG ISLAND SOUND
COLLECTION TEMPERATURE: 19.30 DEGREES C COLLECTION SALINITY: 28.20 PPT
ACCLIMATION: 14 DAYS AT 20 C.
SEDIMENT SOURCE: BARREL OR COLLECTION/JAR NUMBER
SOLID REFERENCE: III/13,14 SOLID BRH: EE/1,2
SUSPENDED REFERENCE: III/13-17 SUSPENDED BRH: EE/1-5

SAMPLE NUMBER	EXPOSURE CONCENTRATIONS (1) NOMINAL	MEASURED	OXYGEN MG/L	MEAN LOG(SCE/CHROM+.1)	MEAN SCE/CHROMO
400122	200MG/L REF/REF	217.3 ± 85.9		-0.49	0.32 ± 0.10*
400123	200MG/L BRH/REF	190.1 ± 60.7		-0.44	0.30 ± 0.03
400124	200MG/L REF/BRH	217.3 ± 85.9		-0.60	0.20 ± 0.03
400125	200MG/L BRH/BRH	190.1 ± 60.7		-0.54	0.24 ± 0.05
400150	MMC			0.10	1.73 ± 0.98

* Standard error of the mean.

TABLE B4

LABORATORY WORM DATA SHEET

COE/ERLN FVP

STUDY PLAN: 4   INVESTIGATOR: PESCH ET. AL.

EXPERIMENT DESCRIPTION: SUSPENDED   DATE OF TEST: 830920

EXP. NUMBER: 2 (Table 2)   SPECIES: NEPHTYS INCISA

** EXPERIMENTAL CONDITIONS **

TEMPERATURE: 20.60 DEGREES CENTIGRADE   RANGE: 19.80 - 22.00
SALINITY: 30.70 PARTS PER THOUSAND   RANGE: 30.00 - 31.80
EXPOSURE DURATION: 10 DAYS
PHOTOPERIOD: 12 HOURS
FLOW RATE: 32 MLS/MIN   VOLUME ADDITIONS/DAY 61
NUMBER OF ANIMALS/REPLICATE: 21   NUMBER OF REPLICATES/TREATMENT: 1
ANIMAL'S LIFE STAGE: JUVENILE   AGE: DAYS
SIZE: +/- MG DRY WT   RANGE: 1.184-1.755 MG
CONTROLS: 200MG/L REF/REF
FOOD: PRAWN FLAKE SUSPENSION
ANIMAL SOURCE: SOUTH REFERENCE SITE, LONG ISLAND SOUND
COLLECTION TEMPERATURE: 21.60 DEGREES C   COLLECTION SALINITY: 29.20 PPT
ACCLIMATION: 5 DAYS AT 20 C.

SEDIMENT SOURCE: BARREL OR COLLECTION/JAR NUMBER
SOLID REFERENCE: III/25   SOLID BRH: EE/17
SUSPENDED REFERENCE: III/6,7,36   SUSPENDED BRH: EE/8,10,14,23,*

SAMPLE NUMBER	EXPOSURE CONCENTRATIONS (1) NOMINAL	MEASURED	OXYGEN MG/L	MEAN LOG(SCE/CHROM+.1)	MEAN SCE/CHROMO
400134	200MG/L REF/REF	198.7 ± 73.3	6.60	-0.51	0.25 ± 0.03*
400135	200MG/L BRH/REF	225.6 ± 47.5	6.30	-0.41	0.32 ± 0.05
400137	200MG/L BRH/BRH	225.6 ± 47.5	6.00	-0.51	0.23 ± 0.03
400152	MMC			-0.09	0.82 ± 0.12

* Standard error of the mean.

TABLE B5
LABORATORY WORM DATA SHEET
COE/ERLN FVP

STUDY PLAN: 4 INVESTIGATOR: PESCH ET. AL.

EXPERIMENT DESCRIPTION: SUSPENDED DATE OF TEST: 830902

EXP. NUMBER: 3 (Table 2) SPECIES: NEPHTYS INCISA

** EXPERIMENTAL CONDITIONS **

TEMPERATURE: 21.20 DEGREES CENTIGRADE RANGE: 20.50 - 22.50
SALINITY: 30.50 PARTS PER THOUSAND RANGE: 30.00 - 31.00
EXPOSURE DURATION: 10 DAYS
PHOTOPERIOD: 13 HOURS
FLOW RATE: 35 MLS/MIN VOLUME ADDITIONS/DAY 67
NUMBER OF ANIMALS/REPLICATE: 15 NUMBER OF REPLICATES/TREATMENT: 1
ANIMAL'S LIFE STAGE: JUVENILE AGE: DAYS
SIZE: +/- MG DRY WT RANGE: 1.451-4.488 MG
CONTROLS: 200MG/L REF/REF
FOOD: PRAWN FLAKE SUSPENSION
ANIMAL SOURCE: SOUTH REFERENCE SITE, LONG ISLAND SOUND
COLLECTION TEMPERATURE: 20.50 DEGREES C COLLECTION SALINITY: 28.80 PPT
ACCLIMATION: 7 DAYS AT 20 C.

SEDIMENT SOURCE: BARREL OR COLLECTION/JAR NUMBER

SOLID REFERENCE: III/19 SOLID BRH: EE/5,8
SUSPENDED REFERENCE: III/19,21,22 SUSPENDED BRH: EE/7,11,12

SAMPLE NUMBER	EXPOSURE CONCENTRATIONS (1) NOMINAL	MEASURED	OXYGEN MG/L	MEAN LOG(SCE/CHROM+.1)	MEAN SCE/CHROMO
400130	200MG/L REF/REF	211.0 ± 87.2		-0.48	0.34 ± 0.11*
400131	200MG/L BRH/REF	171.3 ± 52.9		-0.19	0.62 ± 0.07
400132	200MG/L REF/BRH	211.0 ± 87.2		-0.40	0.37 ± 0.06
400133	200MG/L BRH/BRH	171.3 ± 52.9		-0.44	0.31 ± 0.04
400151	MMC			0.14	1.83 ± 1.09

* Standard error of the mean.

TABLE B6

LABORATORY WORM DATA SHEET

COE/ERLN FVP

STUDY PLAN: 4 INVESTIGATOR: PESCH ET. AL.

EXPERIMENT DESCRIPTION: SUSPENDED DATE OF TEST: 830816

EXP. NUMBER: 1 (Table 2) SPECIES: NEANTHES ARENACEODENTATA

** EXPERIMENTAL CONDITIONS **

TEMPERATURE: 21.00 DEGREES CENTIGRADE RANGE: 21.00 - 22.00
SALINITY: 30.00 PARTS PER THOUSAND RANGE: 30.00 - 31.00
EXPOSURE DURATION: 13 DAYS
PHOTOPERIOD: 14 HOURS
FLOW RATE: 35 MLS/MIN VOLUME ADDITIONS/DAY 67
NUMBER OF ANIMALS/REPLICATE: 10 NUMBER OF REPLICATES/TREATMENT: 1
ANIMAL'S LIFE STAGE: JUVENILE AGE: 57 DAYS
SIZE: +/- MG DRY WT RANGE: 7.394-12.012 MG
CONTROLS: 200MG/L REF/REF
FOOD: PRAWN FLAKE DIRECTLY TO DISH
ANIMAL SOURCE: CULTURE
COLLECTION TEMPERATURE: DEGREES C COLLECTION SALINITY: PPT
ACCLIMATION:
SEDIMENT SOURCE: BARREL OR COLLECTION/JAR NUMBER
SOLID REFERENCE: III/13,14 SOLID BRH: EE/1,2
SUSPENDED REFERENCE: III/13-17 SUSPENDED BRH: EE/1-5

SAMPLE NUMBER	EXPOSURE CONCENTRATIONS (1) NOMINAL	MEASURED	OXYGEN MG/L	MEAN LOG(SCE/CHROM+.1)	MEAN SCE/CHROMO
400126	200MG/L REF/REF	217.3 ± 85.9		-0.68	0.14 ± 0.02*
400127	200MG/L BRH/REF	190.1 ± 60.7		-0.65	0.16 ± 0 02
400128	200MG/L REF/BRH	217.3 ± 85.9		-0.64	0.17 ± 0.03
400129	200MG/L BRH/BRH	190.1 ± 60.7		-0.71	0.11 ± 0.02
400154	MMC			-0.34	0.44 ± 0.06

* Standard error of the mean.

TABLE B7

LABORATORY WORM DATA SHEET

COE/ERLN FVP

STUDY PLAN: 4 INVESTIGATOR: PESCH ET. AL.

EXPERIMENT DESCRIPTION: SUSPENDED DATE OF TEST: 830922

EXP. NUMBER: 2 (Table 2) SPECIES: NEANTHES ARENACEODENTATA

** EXPERIMENTAL CONDITIONS **

TEMPERATURE: 20.60 DEGREES CENTIGRADE RANGE: 19.80 - 22.00
SALINITY: 30.70 PARTS PER THOUSAND RANGE: 30.00 - 31.80
EXPOSURE DURATION: 10 DAYS
PHOTOPERIOD: 12 HOURS
FLOW RATE: 33 MLS/MIN VOLUME ADDITIONS/DAY 63
NUMBER OF ANIMALS/REPLICATE: 15 NUMBER OF REPLICATES/TREATMENT: 1
ANIMAL'S LIFE STAGE: JUVENILE AGE: 42 DAYS
SIZE: +/- MG DRY WT RANGE: 1.496-5.124 MG
CONTROLS: 200MG/L REF/REF
FOOD: PRAWN FLAKE SUSPENSION
ANIMAL SOURCE: CULTURE
COLLECTION TEMPERATURE: DEGREES C COLLECTION SALINITY: PPT
ACCLIMATION:

SEDIMENT SOURCE: BARREL OR COLLECTION/JAR NUMBER
SOLID REFERENCE: III/25,26 SOLID BRH: EE/17,18
SUSPENDED REFERENCE: III/6,7,36 SUSPENDED BRH: EE/8,10,14,23,*

SAMPLE NUMBER	EXPOSURE CONCENTRATIONS (1) NOMINAL	MEASURED	OXYGEN MG/L	MEAN LOG(SCE/CHROM+.1)	MEAN SCE/CHROMO
400138	200MG/L REF/REF	199.2 ± 73.2	6.60	-0.68	0.13 ± 0.02*
400139	200MG/L BRH/REF	222.4 ± 43.5	6.30	-0.70	0.11 ± 0.01
400140	200MG/L REF/BRH	199.2 ± 73.2	6.40	-0.70	0.15 ± 0.04
400141	200MG/L BRH/BRH	222.4 ± 43.5	6.00	-0.65	0.17 ± 0.05
400155	MMC			-0.35	0.42 ± 0.06

* Standard error of the mean.

TABLE B8
LABORATORY WORM DATA SHEET
COE/ERLN FVP

STUDY PLAN: 4 INVESTIGATOR: PESCH ET. AL.

EXPERIMENT DESCRIPTION: SUSPENDED DATE OF TEST: 830516

EXP. NUMBER: 1 (Table 3) SPECIES: NEANTHES ARENACEODENTATA

** EXPERIMENTAL CONDITIONS **

TEMPERATURE: 21.00 DEGREES CENTIGRADE RANGE: 20.00 - 21.50
SALINITY: 28.00 PARTS PER THOUSAND RANGE: 28.00 - 29.00
EXPOSURE DURATION: 10 DAYS
PHOTOPERIOD: 15 HOURS
FLOW RATE: 100 MLS/MIN VOLUME ADDITIONS/DAY 192
NUMBER OF ANIMALS/REPLICATE: 30 NUMBER OF REPLICATES/TREATMENT: 2
ANIMAL'S LIFE STAGE: JUVENILE AGE: 41 DAYS
SIZE: 2.1 +/- 0.42 MG DRY WT RANGE:
CONTROLS: 25MG/L REF/REF
FOOD: PRAWN FLAKE DIRECTLY TO DISH
ANIMAL SOURCE: CULTURE
COLLECTION TEMPERATURE: DEGREES C COLLECTION SALINITY: PPT
ACCLIMATION:

SEDIMENT SOURCE: BARREL OR COLLECTION/JAR NUMBER
SOLID REFERENCE: II/50 SOLID BRH: LL/25
SUSPENDED REFERENCE: II/49,52 SUSPENDED BRH: LL/25,28

SAMPLE NUMBER	EXPOSURE CONCENTRATIONS (1) NOMINAL	MEASURED	OXYGEN MG/L	MEAN LOG(SCE/CHROM+.1)	MEAN SCE/CHROMO
400118	25MG/L REF/REF	27.7 ± 0.9		-0.75	0.11 ± 0.03*
400119	25MG/L REF/REF	27.7 ± 0.9		-0.78	0.08 ± 0.02
400120	25MG/L BRH/BRH	24.9 ± 1.5		-0.72	0.12 ± 0.02
400121	25MG/L BRH/BRH	24.9 ± 1.5		-0.80	0.07 ± 0.01

* Standard error of the mean.

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